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Yield and Some Macro-Morphological Characters of *Pleurotus pulmonarius* (Fries) Quel. Fruit Bodies Cultivated on HCl-Optimized Oil Palm Bunch Substrate

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Abstract

This study was conducted to study the yield and some macro-morphological characters of *Pleurotus pulmonarius* fruit bodies cultivated on Hydrochloric acid (HCl) optimized oil palm bunch (OPB) substrate. Concentrated HCl was diluted in tap water at 0.1%, 0.2%, 0.3%, 0.4% and were used to induce changes on the initial pH (9.5) of OPB to 8.9, 8.2, 7.9, 6.2 and control (9.1) respectively; after soaking for 48 hrs. One way Analysis of variance (ANOVA) and Correlation test were adopted for data analysis. Mean separation was also done by Duncan Multiple Range Test (DMRT) at probability level of 5%. Results showed that 0.1%, 0.2%, 0.3% and 0.4% HCl treated OPB substrates produced *P. pulmonarius* primordia after 9, 9, 10, 11 and control (12 days) respectively. Results further revealed that 0.4% HCl treated OPB substrate induced the highest (900 g/kg) fruit body yield and Biological Efficiency (90%) while control (493 g/kg and B.E 49.3%) respectively, produced the lowest quantity of fruit bodies. Some macro-morphological characters of harvested fruit bodies revealed that mean cap size (C.Scm) and Weight (wt.g/kg) of fruit bodies were highest (3.83 cm and 3.5 g/kg) in 0.4% HCl treated OPB respectively. Mean Stipe Length (S.Lcm) was highest (2.77 cm) in 0.3% OPB substrate and was significant at $p \leq 0.05$. S.L and C.S of fruit bodies as well as C.S and Wt. were significantly correlated while there was no correlation between S.L and Wt. of fruit bodies. HCl was found as a suitable acid buffer for the optimization of the pH of the highly alkaline OPB for cultivation of *P. pulmonarius* fruit bodies. Oil palm bunch should therefore be adopted in the commercial production of the Oyster mushroom if certified safe for human consumption.

Keywords: *Pleurotus pulmonarius*; Substrate; pH; HCl

Introduction

Mushrooms are the reproductive structures of fleshy macro fungi which grow wild in the tropical and sub-tropical rainforest. They are capable of degrading lignin and hence are found naturally growing on different woody and non-woody agricultural residue regarded as substrates [1]. These substrate materials are usually by products from industries, households, agriculture etc, and are usually considered as wastes [2,3]. These wastes are actually resources in the wrong place at a particular time [4].

pH is an important factor for good production of oyster mushrooms. Most mushrooms grow and perform well at pH near to neutral or slightly acidic at 6.1 and 7.5 respectively [5]. In order to enhance fruit body production, Oyster mushroom growers prefer to use lime ($CaCO_3$) as an alkaline buffer to optimize pH of some acidic substrates [6]. On the other hand, many growers of oyster mushrooms have experienced difficulties in using oil palm bunch as substrate. Achufusi et al. [7] attempted using oil palm bunch as a substrate for the cultivation of *P. ostreatus*, but recorded no mycelium colonization as well as fruit body yield in the substrate and its supplementations. The substrate was contaminated by *Coprinus cinereus* (a competitor mushroom that contaminates mushroom beds). The pH of the substrate was later found to be high at 10.3 and was suggested as the reason for the growth of the competitor-*Coprinus* sp. and no yield of *P. ostreatus*.

In their separate investigations, Tabi et al. [8] and Lau et al. [9] also reported that *P. ostreatus* was unable to grow on palm empty fruit bunch (EFB) alone and this could be due to high alkaline content of the biomass which does not support mushroom growth. This investigation therefore aims to determine the most efficient means of exploiting Oil Palm Bunch as a substrate for Oyster mushroom production.

Materials and Methods

Source of culture

Pure mycelia culture (Spawn) of *P. pulmonarius* was obtained from the science laboratory of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike.

Location of study

The study was conducted in the mushroom house of the department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Abia State Nigeria.

Spawn preparation/multiplication

Spawn of *P. pulmonarius* was prepared using sorghum grains. *Sorghum* grains were washed in 3 changes of water and soaked overnight. The grains were boiled in tap water for 10-15 minute using gas cooker as a local heat source. Grains was also completely drained of water before mixing with 2% (w/w) $CaCO_3$ and 4% $CaSO_4$ to optimize pH and prevent them from clumping respectively as recommended

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by Muhammad et al. [4,10]. Grains were subsequently packed 2/3 in heat resistant transparent bottles, tightly sealed with Aluminum foil held tight with rubber band before sterilizing in an autoclave at 121°C for 30 minutes. After sterilization, bottles were allowed to cool before they were aseptically inoculated with actively growing mycelia of *P. pulmonarius* by grain to grain transfer and incubated in the dark (at 27 ± 2°C) until grains were fully colonized by *P. pulmonarius* mycelium [11,12] (Figure 1).

Deterimination of pH of substrate

The pH of solution containing 5% of sample substrates in 50 ml of distilled water was determined using Jenway 3070 portable automatic temperature compensation digital pH meter, calibrated with buffer 7-4 and 10. The pH value was read on the digital scale.

Substrate preparation

1 kg of Dry OPB substrate was soaked separately in 0.1%, 0.2% and 0.3% and 0.4% HCl solutions made in 1.5 lit. tap water for 48 hrs. Another 1 kg of OPB substrate was also soaked in same amount of tap water and this served as control, according to Colavolpe et al. The pH of each HCl+OPB substrate was determined after 48 hrs of soaking. All the HCl+OPB substrate levels were packed in an improvised metallic drum (IMD) and pasteurized for 2 hrs using industrial gas cooker as a local heat source and allowed to cool over night. Various HCl+OPB substrates were made into 5 replications and packed in 2.5 liter transparent plastic buckets perforated randomly from bottom to the top.

Substrate inoculation

30 g of grain based spawn of *P. pulmonarius* was spread across each 200 g of OPB substrate packed in perforated transparent plastic buckets during inoculation [3]. All inoculated substrates were placed on wooden racks in the culture room and covered during spawn run. Humidity of the cropping room was optimized by constantly flooding with tap water. Substrates were regularly checked to ensure optimum moisture content prior to primordial initiation.

Measurement of morphological characters

Mushrooms growing on substrate of various percentage solutions of HCl were harvested at maturity. Stipe length and cap size were measured in centimeter using meter rule [3]. Fresh weight of individual fruit bodies was also determined using digital weighing balance.

Yield and biological efficiency

Harvested fresh fruit bodies from each 5 replicate levels of HCl treated OPB were weighed using digital weighing balance while Biological efficiency (BE%) was determined using the method of Chang and Miles.

Statistical analysis

Data obtained during the experiment were statistically analyzed using Analysis of Variance (ANOVA), mean separation and tests of significance were carried out by Duncan Multiple Range Test (DMRT) at $p \leq 0.05$ respectively [12].

Results and Discussion

Table 1 shows the effect of different concentrations of HCl acid solution on the pH of OPB and fruiting duration of *P. pulmonarius*. Result shows that the initial pH of OPB was found to be 9.5. Different percentage concentrations of HCl solution altered the pH of OPB in the following order: 0.1% (8.9), 0.2% (8.2), 0.3% (7.9) and 0.4% (6.2), while control was found at pH of 9.1 after soaking for 48 hrs. This shows that concentrated Hydrochloric acid simply acted as an acid buffer capable of optimizing the pH of an alkaline OPB substrate to support the growth of mushroom.

The fruiting duration recorded in this work shows that oil palm bunch has the ability of inducing earlier fruiting of *P. ostreatus* and does not conform with the work of Shah et al. which recorded longer fruiting time for *P. ostreatus* on saw dust, wheat straws and other agro wastes combinations. Khan et al. observed relatively shorter fruiting duration when oyster mushroom on different lignocellulosic substrates. They recorded that pin head formation started 7-8 days and matured between 10-12 days of substrates inoculation. This could be that the substrates used contained more nutrients that supported early primordial formation.

During spawn run, all the HCl+OPB substrates including the control were colonized by mycelium (Sudirman et al.). Although, *Coprinus cinereus,* a contaminant mushroom was seen growing on control and other HCl+OPB substrates after 6 days, except 0.4% HCl+OPB. *C. cinereus* is a major contaminant in OPB when used in mushroom cultivation, even though pasteurization time is increased. The result also proves that *C. cinereus* grows better in a high alkaline medium as 0.4% HCl solution was able to reduce the high alkalinity (pH 6.2) of the OPB as well as *C. cinereus* contamination of the substrate.

Duration of primordia initiation shows that 0.1%+OPB took 8 days, 0.2%+OPB (9 days), 0.3%+OPB (10 days) and 0.4% OPB (11 days) to produce mushroom primordia while control took the longest duration of 12 days. This result conforms to assertion of Khan et al. which maintained that most mushrooms grow better in pH approximately near to neutral i.e., 7.0, but differ with those of Quimio; Quimio and Sardsud which noted that fruit bodies of *P. ostreatus* grown on various substrates emerged within 3-4 weeks after substrate inoculation. According to them, various factors such as pH, temperature, nature of substrate and method of pasteurization can determine the fruiting time of oyster mushrooms.

Table 2 shows the effect of various HCl solution concentrations on the yield and B.E (%) of *P. pulmonarius* fruit bodies cultivated on OPB substrate. From the result, 0.4% HCl+OPB gave the highest (900 g) fruit body yield and B.E (90.00%), followed by 0.3%+OPB (820 g), 0.2%+OPB (774 g) and 0.1%+(569 g) while control gave the lowest (493 g) with B.E of 49.30%. This result shows that as the concentration of Hydrochloric acid increased increased from 0.1- 0.4%, yield and biological efficiency of *P. pulmonarius* increased from 569.00 g (56.9%)-900.00 g (90.00%) while control 493.00 g (49.3%) respectively. This trend was due to *Coprinus cinereus* which contaminated the substrate, from control to 0.3% Hcl OPB (Achufusi). Tabi et al. and Lau et al. [9] had similar experience and reported that *P. ostreatus* was unable to grow on palm empty fruit bunch (EFB) alone and this could be due to high alkaline content of the biomass which does not support mushroom growth. Report of Khan

HCl+OPB (%)	pH of OPB	Fruiting duration (days)
OPB before HCl treatment	9.5	_
0.1+OPB	8.9	9
0.2+OPB	8.2	9
0.3+OPB	7.9	10
0.4+OPB	6.2	11
Control	9.1	12

OPB=Oil Palm Bunch, HCl=Hydrochloric acid

Table 1: Effect of Percentage HCl Concentrations on the pH of OPB and Fruiting Duration of *P. pulmonarius*.

HCl+OPB (%)	1st flush g/kg	2nd flush g/kg	Total Yield g/kg	D/wt Substrate/g/kg	BE (%)
0.1+OPB	490	79	569	1000	56.90
0.2+OPB	604	170	774	1000	77.40
0.3+OPB	626	203	829	1000	82.90
0.4+OPB	604	296	900	1000	90.00
Control	268	225	493	1000	49.30

OPB=Oil Palm Bunch, HCl=Hydrochloric acid. D/wt=dry weight

Table 2: Effect of Percentage HCl Solution Conc. on the Yield (gm) and Biological Efficiency (BE%) of *P. pulmonarius* fruit bodies on OPB substrate.

HCL+OPB (%)	S. L (cm)	C. S (cm)	Wt (g/kg)
0.1 HCl+OPB	2.01c	3.17b	2.27b
0.2 HCl+OPB	2.67ba	3.48ba	2.68b
0.3 HCl+OPB	2.77a	3.72ba	2.79b
0.4 HCl+OPB	2.49bac	3.83a	3.59a
Control	2.23bc	2.49c	1.54c

OPB=Oil Palm Bunch, HCl=Hydrochloric acid. Values are means of 3 replicates, means with the same letters are not significantly different at p>0.05.

Table 3: Effect of Percentage HCl Solution Treated Substrate on S.L, C.S and Wt. of Fruit Bodies.

	SL	CS	WT
SL	-	0.48^{**}	0.36^{ns}
CS	-	-	0.59^{**}
WT	-	-	-

Values are means of 5 replicates. *Significant Correlation; **Highly Significant Correlation; ns: No significant correlation. SL: Stipe length; CS: Cap size; WT: Weight.

Table 4: Correlation Effect HCl+OPB Substrates on S.L, C.S and WT of *P. pulmonarius* Fruit Bodies.

Figure 1: (a) Plate 1: Oil Palm Bunch (OPB) Substrate Plate. **(b)** Plate 2: *P. pulmonarius* fruit bodies emerging from OPB.

et al. [5] had earlier supported this observation by stating that most mushrooms grow and perform well at pH near to neutral or slightly acidic at 6.1 and 7.5 respectively.

Table 3 represents the effect of percentage HCl solution concentrations on the S.L (cm), C.S (cm) and Wt.(g/kg) of *P. pulmonarius* fruit bodies cultivated on OPB. 0.3% HCl treated OPB substrate supported slightly high (2.77 cm) S.L than others while 0.1% gave seemingly the lowest (2.01 cm) S.L. All the percentage HCl treated substrate including control did not show a significant difference on the stipe length of *P. pulmonarius* fruit bodies at p>0.05 supporting the work of Okwulehie et al. [13]. Cap size (C.S) was smallest (2.49 cm) in the mushroom produced the control OPB substrate. But the largest (3.83 cm) C.S was observed in the 0.4%HCl treated OPB substrate. Individual mushroom weight shows an increased gradient from control to 0.4% HCl treated OPB and were not significant at p>0.05. Okwulehie et al. [13] observed that *Pennisetum* straw significantly increased the stipe length of *P. ostreatus*, followed by *A. gayanus* straw, *Oryza* straw. They also note that *Panicum* straw causes a reduction of the cap diameter. These findings revealed that substrate has significant effect on the macro-morphological characters of oyster mushrooms. Okoi et al. [14] maintained that different substrates have affect pileus diameter, stipe height and stipe girth. Apart from substrate Hydrochloric acid, Ogbo et al. reported that crude oil has significant effect macro morphological characteristics such as pileus diameter, stipe height, stipe girth, fresh weigh and weight of mushroom fruit bodies. One of the factors responsible for the production of healthy fruit bodies in mushroom industries is the absence of contaminants in the substrates [6]. Steam pasteurization may have ensured more effective pasteurization of the substrates than other techniques studied.

Table 4 represents the result of the relationship between S.L (cm), C.S (cm) and wt.(g/kg) of *P. pulmonarius* fruit bodies cultivated on HCl+OPB substrates. The result shows that there is high level of relationship between S.L and C. S at Rho=0.48** but shows no significant relationship between S.L and Wt. (0.36 ns). C.S also shows a high level significant relationship with weight of the fruit bodies (Rho=0.59**). This simply means that S.L of *P. pulmonarius* fruit bodies with respect to the assay was highly related to C.S while C.S does same to Wt. of the mushroom [15-20].

Conclusion

In an experiment to determine the effect of HCl on OPB substrate for the cultivation of *P. pulmonarius* fruit bodies, it was found that HCl could be used as a suitable acid buffer to optimize the pH of alkaline-oil palm bunch (OPB) for the cultivation of *P. pulmonarius* fruit bodies. The pH of the raw OPB was at alkaline/pH value of 9.5 and in some cases 10.3 or more. Increase in HCl solution concentration from 0.1 - 0.4%, led to an increase in the acidity of OPB substrate from pH of 8.9 to 6.2 and increase in *P. pulmonarius* fruit body production while the OPB substrate used as control remained at 9.1. This shows that HCl is a good acid buffer to adjust the pH of OPB before it could be used for oyster mushroom.

Recommendations

Mushroom growers should harness HCl+OPB especially at 0.4% level in the cultivation of *P. pulmonarius* fruit- bodies. Percentage HCl solution should be increased to 0.5 or 0.6% to know if they would achieve better yield, Biological Efficiency and other growth parameters investigated in this research Further studies should aim to determine the consumption safety of *P. pulmonary* produced from HCl+OPB substrate. Finally, mushroom scientists should identify other cheaper alternative natural or organic acid buffer that would substitute synthetic acid. This could eliminate the irrational safety fear that may arise in the consumption of this mushroom

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